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TECHNICAL REPORT

Study of the Antitoxic Effect of Unithiol on Cystamine in Dogs

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It was shown in experiments using a canine model that intramuscular administration of unithiol (monohydrate sodium salt of 2,3-dimercapto-1-propansulfonic acid, 50 mg/kg) before cystamine (cystamine dihydrochloride, 100 mg/kg, calculation on the salt, intragastric administration) decreases the toxic effect of this radioprotector. The frequency of manifestations of the toxic effects of cystamine (change of behavior, vomiting reaction, decrease of body temperature and so on) is not decreased, but the severity of expression and duration are decreased. The period of agitation induced by cystamine is shortened, and the period and expression of vomiting induced by this preparation are decreased. Respiratory frequency and condition of the heart are less changed (according to EKG data).

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CONVERSION TABLE

Conversion factors for U.S. Customary to metric (SI units of measurement)

MULTIPLY TO GET	BY BY	TO GET DIVIDE
angstrom	1.000 000 x E-10	meters (m)
atmosphere	1.012 25 x E +2	kilo pascal (kPa)
bar	1.000 000 x E + 2	kilo pascal (kPa)
barn	1.000 x E – 28	meter^2 (m^2)
British thermal unit (thermochemical)	1.054 350 x E + 3	joule (J)
calorie (thermochemical)	4.184 000	joule (J)
cal (thermochemical)/cm^2	4.184 000 x E-2	mega joule/m^2 (MJ/m^2)
curie	3.700 000 x E + 1	giga becquerel (GBq)*
degree (angle)	1.745 329 x E – 2	radian (rad)
degree (Fahrenheit)	Tk = (t +459.69)/1.8	degree kelvin (K)
electron volt	1.602 19 x E – 19	joule (J)
erg	1.000 000 x E – 7	joule (J)
erg/sec	1.000 000 x E – 7	watt (W)
foot	3.048 000 x X-1	meter (m)
foot-pound-force	1.355 818	joule (J)
gallon (U.S. liquid)	3.785 412 x E – 3	meter^3 (m^3)
inch	2.540 000 x E -2	meter (m)
jerk	1.000 000 x E + 9	joule (J)
joule/kilogram (J/kg) (absorbed dose)	1.000 000	Gray (Gy)**
kilotons	4.183	terajoules
kip (1000 lbf)	4.448 222 x E + 3	newton (N)
kip/inch^2 (ksi)	6.894 757 x E +3	kilo pascal (kPa)
ktap	1.000 000 x E +2	newton-second/m^2 (N-s/m^2)
micron	1.000 000 x E – 6	meter (m)
mil	2.540 000 x E – 5	meter (m)
mile (international)	1.609 344 x E + 3	meter (m)
ounce	2.834 952 x E – 2	kilogram (kg)
pound-force (lbf avoirdupois)	4.448 222	newton (N)
pound-force inch	1.129 848 x E – 1	newton-meter (N*m)
pound-force/inch	1.751 268 x E + 2	newton-meter (N/m)
pound-force/foot^2	4.788 026 x E – 2	kilo pascal (kPa)
pound-force/inch^2 (psi)	6.894 757	kilo pascal (kPa)
pound-mass-foot^2 (moment of inertia)	4.214 011 x E – 2	kilogram-meter^2 (kg*m^2)
pound-mass/foot^3	1.601 846 x E + 1	kilogram/m^3 (kg/m^3)
rad (radiation absorbed dose)	1.000 000 x E – 2	Gray (Gy) **
rem (roentgen equivalent man)		Sievert (Sv) ***
roentgen	2.579 760 x E – 4	coulomb/kilogram (C/kg)
shake	1.000 000 x E – 8	second (s)
Slug	1.459 390 x E + 1	kilogram (kg)
Torr (mm Hg, 0 degrees C)	1.333 22 x E – 1	kilo pascal (kPa)

* *The Becquerel (Bq) is the SI unit of radioactivity: 1 Bq = 1 event/s.*

** *The Gray (Gy) is the SI unit of absorbed radiation.*

*** *The Sievert (Sv) is the SI unit of dose equivalent.*

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ABSTRACT

It was shown in experiments using a canine model that intramuscular administration of unithiol (monohydrate sodium salt of 2,3-dimercapto-1-propansulfonic acid, 50 mg/kg) before cystamine (cystamine dihydrochloride, 100 mg/kg, calculation on the salt, intragastric administration) decreases the toxic effect of this radioprotector. The frequency of manifestations of the toxic effects of cystamine (change of behavior, vomiting reaction, decrease of body temperature and so on) is not decreased, but the severity of expression and duration are decreased. The period of agitation induced by cystamine is shortened, and the period and expression of vomiting induced by this preparation are decreased. Respiratory frequency and condition of the heart are less changed (according to EKG data).

FOREWORD

Like all drugs, radioprotectors are toxic at sufficiently high concentrations. It is known that the toxic effects of thiol radioprotectors, in both dogs and humans, include asthenia (weakness), nausea, vomiting, loss of work capacity, and other effects. In humans postural hypotension and dizziness also occur. In healthy troops or civilian first responders exposed to radiation such effects clearly would prolong the time of exposure, increase the risk of accidents, or impair their ability to perform their duty. As a result, the beneficial effects of the radioprotector could be diminished or even reversed. A major aim of researchers in this field is therefore to decrease toxicity while maintaining efficacy.

The authors of this report have previously shown (22) that unithiol, in combination with the radioprotector cystamine, significantly reduced the toxicity of the latter, as measured by $LD_{50/30}$ in mice. If the dose of cystamine was subsequently doubled, and unithiol administered, the dose protection factor could be increased from 1.4 to 1.8. While such an approach might not be useful in a military operational situation, clearly the radioprotective effects of this particular radioprotector were not reduced. The next step was to test the antitoxic effects of adding unithiol to cystamine in a physiologically and behaviorally more complex animal model. This report documents the results of this experiment.

Although cystamine related toxic effects were not eliminated, their expression and duration were reduced. More work should be done along the lines of this study's directions. One approach would be to alter the dose and ratios of this particular combination of radioprotective agents. The ability of other dithiols to ameliorate the effects of other radioprotectors should also be studied.

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SECTION 1

STUDY OF THE ANTITOXIC EFFECT OF UNITHIOL ON CYSTAMINE IN DOGS

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Beginning with the well known works of Patt (34a), Bacq (2a, 2b), and Doherty (20a), the study of radioprotectors has had theoretical as well as practical significance. Most attention has been given to sulfur-containing compounds which provide effective protection against both low LET and neutron radiation to animals of various species. Positive experience of use of sulfur-containing protectors has been accumulated in the clinical practice of radiotherapy of cancer. Moreover, these compounds are beginning to attract the attention of virologists, immunologists and geneticists (24, 25). However, widespread clinical use of the sulfur-containing preparations is restricted by their toxicity. In fact, the sulfur-containing protectors show radioprotective effect at doses which are close to toxic level, and the therapeutic index of these compounds (minimal toxic dose/minimal radioprotective dose) in experiments in mice is not large - less than 3 (11).

The toxic effects of the aminothiol radioprotectors cysteamine (MEA), cystamine, and AET (aminoethylisothiouranium•Br•HBr) are varied and are variously manifested in animals of different species. After a single administration they are expressed in the form of depression, decreased movement and postural changes, tremor, and spasms in mice and rats. An intravenous administration of MEA in dogs (200 mg/kg) causes restlessness, which is quickly changed to flaccidity, multiple episodes of vomiting, hypersalivation, tachycardia, and weak pulse for four to eight hours. These symptoms persist, although they are less pronounced, when the dose is halved (45). Also noted are shortness of breath, convulsive twitching of extremities, decreased appetite (35), spasms, ataxia, rocking motions of the front half of the body, loss of sensitivity to painful stimuli, and diarrhea (34).

In addition to the studies outlined above the authors of a detailed study of AET in various species of animals noted that tenesmus, defecation, and mydriasis are observed in dogs (5).

The thiol protectors AET, MEA, and cystamine cause initial agitation with subsequent depression, somnolence and decrease of muscular tone, salivation, and vomiting in monkeys (Macaca mulatta, Macaca rhesus). In the case of dogs administration of MEA and cystamine 150 mg/kg induces a shock-like condition and spasms. Cystaphos, which is similar to these protectors, is less toxic, especially with oral administration. However, its radioprotective doses cause deep depression and vomiting (19, 23, 37).

Disorders of the cardiovascular system are a constant toxic effect of aminothiol preparations. Intravenous administration of AET in dogs induces hypertension that is due to increased peripheral resistance. If the drug is administered slowly hypertension gives way to hypotension. Development of refractoriness of the myocardium to vagal stimulation was noted as well (26). In addition to the changes of blood pressure described, decrease of the QRS complex and the S and T waves, inversion of the ST segment and, in some instances, complete inversion of the QRS complex were found on electrocardiography (EKG) (8).

Under the same conditions of administration, MEA causes persistent hypotension, which is

also explained by varying peripheral resistance as well, but in the opposite direction (34). The hypotensive effect of MEA and cystamine are outlined not only in dogs but in animals of other species (31, 36, 39, 47). They also cause decrease of blood pressure in cats and monkeys (37, 39), and cystaphos in monkeys causes bradycardia (19).

All enumerated side effects of the aminothiols arise from use of doses close to radioprotective ones, and exceeding these doses causes death of animals by cessation of respiration (43).

Clearly, the aminothiol protectors are toxic in humans as well. True, it has been shown that the side effect of the preparation is not noted if cystamine is administered in small doses per os (orally) (for example, 0.2 - 0.4 g cystamine in a single dose) (13). However, the authors of this presentation did not study the condition of the cardiovascular system. Other authors have found that, given the same conditions of administration of cystamine, blood pressure and peripheral resistance are decreased, stroke volume of the heart is increased, and the basic metabolism is increased as well. Respiration is changed in persons with hypertensive disease (27). The above mentioned doses of cystamine are essentially less than optimal radioprotective doses in humans as determined by extrapolation of results from experiments in animals.

AET causes an expressed toxic effect in humans as well. Nausea, belching, vomiting and hyperemia of the face and skin of the thorax are noted upon intravenous administration. Nausea and vomiting, small decrease of blood pressure, vertigo, increased sweating, and sleepiness appear upon administration of the preparation in doses of 750-1,000 mg per os. The authors noted that these effects are caused at doses of AET which do not protect the skin against the effects of radiation and do not prevent epilation (12).

Mechanisms of the toxic effect of the aminothiol preparations are various. Judging from the character of the symptoms, the toxic effect of aminothiol radioprotectors are caused primarily by their influence on the central nervous system (CNS). Changes of behavior attributable to the aminothiol protectors are associated with disturbance of the functional activity of cortex. It was demonstrated, using the method of conditioned reflexes, that MEA even in small doses decreases excitation of the cortex in rat. The subcortex is depressed as well; unconditioned reflex activity deteriorates (3). The results of electroencephalography (EEG) in rabbits and cats agree well with these data: in these animals bioelectric activity of the sensorimotor zone of the cortex and reticular structure of middle brain was decreased (7, 16). Administration of MEA and mercaptopropylamine to rabbits leads to decrease of free oxygen in the brain by 25 - 30% (6). Radioprotective doses not only depress the cerebral cortex and subcortex of rats, but also decrease their spinal cord reflexes (3).

Changes of the functional condition of nervous centers can be determined by changes of circulation of the blood and respiratory activity. In particular, the experiments demonstrated that administration of only 75 mg/kg of MEA in the vertebral artery of cats caused rapid acceleration of the frequency of respiration and hypotension, which was more pronounced than from intravenous administration of the same dose of the protector (39). However, the cause of changes of the vegetative function is not only disturbance of nervous centers but changes of reflex regulation as well. Thus MEA changes excitability of the chemoreceptors of the carotid sinus area, the most important reflexogenic zone in the system of regulation of breathing and blood circulation (38). Change of heart activity (bradycardia) and breathing in rabbits and cats under the influence of MEA takes place involving the carotid sinus and vagal reflexes. The subsequent hypertension is associated with excitation of spinal vessel motor centers (31).

It was found that depression of ganglionar transmission by the aminothiol preparations may

play a role in disturbance of vegetative functions (20, 21). Finally, humoral effects of the thiol protectors and the hormonal agents they induce take part in changing the tone of blood vessels (1, 2, 34). Here direct influence of the aminothiols on the blood vessel walls takes place (32, 47), and, in the case of MEA, a sympatholytic effect (32) and intensified formation of histamine (29). Administration of AET as a humoral factor increases blood vessel wall tone; blood vessel wall tone may also be influenced by adrenaline and noradrenaline, induced by administration of the protector, as well as by a direct effect of the protector itself (5).

Vomiting and changes of intestinal peristalsis under the influence of MEA, cystamine, and AET are associated with the effect of the protectors on the CNS, as well as with a local effect due to reflexes from receptors in the mucous membrane of the stomach and intestines (32, 33, 41, 44, 47).

By this means, one can see that the particular mechanisms of influence on the system of blood circulation are different between MEA on the one hand and AET on the other hand. However, there is doubtless a principal similarity in the mechanisms of toxic effect of all the protectors named. The basis for this similarity is disturbance of the functional condition of the central nervous system, disturbance of the reflex regulation of vegetative functions, and a direct effect of the protectors on several organs. These are associated with effectors involved in the reaction from hormonal agents.

The necessity of decreasing the side effects of aminothiol radioprotectors described above was determined almost from the time of discovery of their radioprotective effect.

One way of decreasing the toxic effect of the aminothiol preparations is to change the method of their administration to the organism: by oral administration they show less toxic effect than when administered parenterally. Unfortunately, not all the aminothiol protectors can be administered this way. However, if it is possible (as, for example, in the case of AET and cystamine), it substantially decreases the toxic influence of a preparation. So, the LD50 is 392 mg/kg with intraperitoneal administration of cystamine and 1392 mg/kg with per os administration (22). There are data to the effect that the toxicity of a preparation is decreased if it is administered for a few days in an increasing dose, beginning from subtoxic levels and concluding at obviously toxic doses (23).

Decrease of toxicity of MEA and cystamine may also be achieved as well by means of appropriate selection of the acid with which the protector (a base) forms a salt: various salts of the same protector are not equally toxic. So, the hydrochloride of MEA is the most toxic in terms of its depressive effect on spinal cord reflexes in cats, and the ascorbinate is toxic only to a minimal degree. The hydrochloride of MEA is the most toxic in terms of its hypotensive effect in cats and rabbits, and the ascorbinate is least toxic of all; the salicylate occupies an intermediate position (4). The lethal effect of the lactate of MEA is greater than the same dose of the hydrochloride, and the latter is more toxic than ascorbinate; the tartrate and bitartrate of MEA, in general, do not cause the death of animals at the same dose (51). However, the indicated differences are not very large, and the change of one acid residue to another cannot solve the problem of toxicity of the aminothiol protectors. Synthesis of phosphoro derivatives of the aminothiols - cystaphos and especially WR-2721 - is an important step in this direction. But their use is also accompanied by side effects in animals of all species, in monkeys as well as in man (9, 19, 46). This demands action for decrease of the toxicity of these phosphoro derivatives of the aminothiols.

One important way of solving the problem of toxicity of the aminothiols is a combination of the aminothiol preparations with various chemical agents (22, 49). An important role of thiol compounds is to cause changes of the condition of the central nervous system; although this causes

toxic effects, the intended use of the majority of these agents has a favorable influence on the CNS. Indeed, the narcotic and anticonvulsive preparations pentobarbital, luminal, librium, and benzonal ameliorate the toxic effect of cystamine: the first of these enumerated preparations decreases salivation, vomiting and spasms in dogs from administration of cystamine (34), and benzonal decreases mortality in mice when used with cystamine (18). Caffeine at a dose of 40 mg/kg acts in a similar fashion regarding WR-2721 and WR-3689 (28, 50). Preparations which excite the nervous system, such as strychnine, corazol, phenamine, and even, in relatively large doses, caffeine increase the toxic effect of cystamine and cystaphos, promoting their lethal effect (42). Besides neurotropic drugs, ACTH and hormones of the adrenal cortex also increase tolerance to cystamine (40, 42). Zinc, copper, and selenium act similarly with respect to WR-2721. In addition, they increase the radioprotective efficacy of MEA, AET, and WR-2721 (10, 17, 48, 49, 50).

Stability of an organism against the toxic effects of aminothiol protectors can also be attained depending on how they are combined with one another. So, the toxicity and side effects of the combinations WR-2721 and cystaphos, AET and 2-mercaptopropylamine, MEG and MEA, cystamine and AET, cystamine and cystaphos are less pronounced than when each agent is administered alone (14,18,28,30,50).

As shown, the combination of cystamine, MEA or AET with unithiol (sodium salt of 2, 3-dimercapto-1-propansulfonic acid) significantly increases the stability of mice and rats to the toxic effects of these radioprotectors. The LD50 increases under the influence of unithiol by 40%; that is substantially more than with any of the preparations mentioned above, alone or combined with one another. The increase in stability to cystamine or AET allows one to increase doses of the latter, and, as a consequence of this, the radioprotective effect as well. We found that there is an optimal ratio of unithiol and the aminothiol protectors to increase the stability of the organism to the toxic effect of the latter (15, 22).

In connection with this it is expedient to research the antitoxic effect of unithiol concerning one of the most effective radioprotectors, cystamine, in dogs - animals closer in biological structure to man than rodents.

SECTION 2

MATERIALS AND METHODS

For this experiment twenty mongrel adult male dogs were used. Distribution of animals by weight was the same in both groups. They had the standard vivarium diet (meat, milk, bread, groats, vegetables, vitamins). Drinking water was constantly available. The first (control) group of ten dogs was given cystamine, and the second (experimental) group of ten dogs was given unithiol and cystamine in solution. Cystamine was given through a tube into the stomach. Per os administration allows better simulation of the actual situation of use of this preparation as a tablet in humans, rather than via intravenous (IV) or intraperitoneal (IP) administration. Unithiol was given intramuscularly 10-15 minutes before cystamine. It was shown earlier that this way of unithiol administration provides an increase in stability to the effects of cystamine administered into the stomach (22). The dose of cystamine hydrochloride was 100 mg/kg (calculation on the salt), and the dose of sodium salt of unithiol was 50 mg/kg. Such a ratio of doses of cystamine and unithiol (1 equivalent of cystamine to 1/2 equivalent of unithiol) provides the most pronounced antitoxic effect of unithiol (22).

The preparations of cystamine dihydrochloride and monohydrate of sodium salt of 2,3-dimercapto-1-propanesulfonic (Unithiol) were purchased from commercial suppliers and used without further purification. The purity of unithiol was not less than 98 % as shown by SH-group spectrophotometric analysis with Ellman reagent. The purity of cystamine as detected by SH-group spectrophotometry after reduction of the disulfide bond by $NaBH_4$ lay within the range of $99 \pm 3\%$.

The toxic effect of cystamine and the changing resistance of the animals to it were judged by observing their behavior (agitation and depression), their posture, motor activity, tremor, salivation, vomiting, defecation, respiratory rate, pulse rate, electrocardiographic (EKG) data, temperature, and physical working capacity (ability to tolerate dynamic physical loading of high intensity).

Physical working capacity was evaluated using a tredban (treadmill with a designated speed, on which the dog is forced to run). The dogs were trained to run by tredban for two weeks, using one 40-minute training run everyday. Pulse and breathing rates would increase until the pulse rate had increased by 20-30 beats per minute (bpm) above the initial resting level. After awhile the respiratory rate would begin to decrease. When it had decreased by 5-6 breaths per minute, relative to the maximum achieved level, this indicated arrival at the aerobic-anaerobic threshold (AAT), which occurs at an oxygen debt of more than 2-3 liters.

Such debt cannot be cancelled without involving conditions of anaerobic oxidation and corresponds to accumulation of lactic acid in blood to 5 mM/l. A continuation of the loading leads to fatigue and to irreversible changes in various organ systems due to hypoxia (39a).

On the test day the animals were made to run at the same speed they did during the two week training period. When the pulse and respiratory rates changed to indicate achievement of the AAT, the run was stopped and the time recorded. This was expressed as a percentage of the time it took the animal to achieve AAT during the training period. The time to complete recovery of the pulse and respiratory rates was also recorded.

Pulse rate was determined by EKG, and respiratory rate by using a transducer fixed on the animal's thoracic cage. Changes of resistance to electric current in the transducer during respiratory movement were recorded using a polygraph.

The measurements of all the characteristics of the animals' condition were carried out before administration of the preparations and at various periods after their administration. The time of appearance of changes of the characteristics being studied, their intensity and duration were recorded. The number of vomiting episodes was recorded. All experiments were begun at 10:00 am. The dogs were not fed on the day of the experiment prior to testing.

The results of the experiments were processed using Student's criterion and non-parametric criteria of signs.

SECTION 3

RESULTS

A short time after administration of cystamine into the stomach, excitation, unrest, abundant salivation, and then repeated paroxysms of vomiting were noted in dogs of both groups. Urination and defecation took place in some instances. In some of the dogs of the first group the agitation was superceded by depression after 30-40 minutes, and an active posture followed by a passive one: dogs were lying down with their head on their paws. Depression was not observed in animals of the second group.

Rectal temperature in both groups of animals decreased under the influence of cystamine. Some dogs developed tremor. Cystamine causes tachypnea, which was significant in the first group. Respiratory rate increased by 35% by the 30th minute, and by the 60th minute the increased rate still persisted in 30% of the animals in the first group. In the second group these changes were notably decreased by the 30th minute and disappeared almost completely by the 60th minute.

The pulse rate changed as well, although not as pronounced as the respiratory rate. The pulse rate was increased in both groups at the 30th minute; to 15% in the first group and to 20% in the second (the distinctions were not significant). The pulse rate was back to normal by the 60th minute. All these changes described above were eliminated in the first group of animals by one hour, and even earlier in the second group. After two hours the condition of all dogs in both groups was no different from the starting condition.

Cystamine-induced changes of the heart condition were detected in several dogs from both groups by electrocardiographic studies. All these conditions are reflected in the following tables.

Table 1. Frequency of Certain Toxic Manifestations.

Indicators	Cystamine	Cystamine + Unithiol
Changes of Behavior:		
Agitation	10	10
Change of agitation to depression	3	-
Increase of Salivation		
Strong	7	3
Weak	3	7
Posture		
Active	7	10
Passive	3	-
Tremor	3	3
Vomiting	10	8
Defecation and Urination	6	2
Decrease in Rectal Temperature	10	10
Changes in Physical Work Capacity	7	4
EKG Changes	6	2

According to the non-parametric criteria of signs, all differences between the groups were not

statistically significant. As shown in Table 1, administration of unithiol before cystamine in dogs has no influence on the frequency of manifestation of toxic effects of the radioprotector. The toxic effects of cystamine were observed in all dogs of both groups, regardless of whether cystamine was administered alone or in combination with unithiol. The differences in frequency of appearance of these and other symptoms of the toxic effects of cystamine in dogs of both groups were not statistically significant.

However, statistically significant differences in evaluation of the expression and duration of several toxic effects of the protector between animals of the two groups were revealed, shown in Table 2 and Table 3.

Table 2. Magnitude of Toxic Effects.

Indicators	Cystamine	Cystamine + Unithiol
Change of Behavior		
Time from exposure to agitation (minutes)	9.9±1.36	11.2±1.92
Duration of period of agitation (minutes)	24.3±4.5	10.4±2.16*
Decrease in Body Temperature (degrees C)	1.0±0.28	0.7±0.07
Pulse Rate (% of initial value)		
After 30 minutes	115.2±17.7	120.0±7.68
After 60 minutes	94.3±6.5	103.5±4.7
Respiratory Rate (% of initial value)		
After 30 minutes	185.5±27.6	115.0±14.03**
After 60 minutes	130.8±31.0	104.9±26.5
Physical Work Capacity, or Time to Onset of the Aerobic-Anaerobic Threshold (% of initial value)	-	-
Within 1 hour after administration of the preparation	96.9±18.2	98.8±20.4
Within 3 hours after administration of the preparation	76.2±11.2	82.5±10.8
Time to Complete Recovery (minutes)	59.4±3.2	28.2±3.7*

** p< 0.02, **p< 0.05*

This primarily concerns changes of behavior: although the latent period till onset of agitation in dogs of both groups is practically the same - 9.9 and 11.2 minutes, the duration of agitation in dogs

of the second is half as long - 10.4 instead of 24.3 minutes. After the period of agitation dogs of the second group quickly return to their initial condition, keeping an active posture. They can hardly be distinguished in appearance from the animals which are not exposed to any influence. In contrast, agitation gave way to depression in dogs of the control group and their pose became passive. True, the differences in frequency of cases of agitation and depression are statistically not significant. If all the changes are considered, which are observable in the condition and behavior of the dogs, the time of return to the initial condition in animals of the control group is an average of 59.4 minutes, whereas in animals of the experimental group it is 28.2 minutes.

The administration of unithiol does not affect the decrease of temperature induced by cystamine; the temperature reaction is the same in animals of both groups. The differences of the temperature changes are small and are not statistically significant, as illustrated in Table 2. Unithiol does not affect the duration of the latent period and intensity of salivation as well; the former is equally short and the latter is equally intense in all dogs which received cystamine, no matter whether unithiol was previously administered to them or not, as shown in Table 3.

Table 3. Influence on condition of the digestive system.

Reaction	Cystamine	Cystamine + Unithiol
Salivation		
Latent period, minutes	6.4±1.6	6.6±1.7
Emesis		
Latent period, minutes	5.1±1.8	18.6±2.7
Average number of episodes of emesis	4.2±1.3	2.1±0.5*
Duration of Period of Emesis (minutes)	48.4±12.9	18.6±1.38*

** $p < 0.01$*

Influence of unithiol on the speed of appearance of emesis from cystamine is not observed; the latent period of this reaction in dogs of each group is statistically indistinguishable (Table 3). However, the intensity of the emetic response to cystamine in dogs which received unithiol prior to receiving the protector is decreased. This shows itself as a decrease in the number of episodes of emesis in dogs of the second group, as well as a shorter duration of the period of emesis. The corresponding indicators are decreased more than twice as much in dogs of the second group as compared with the control group (Table 3). Note (Table 1) that vomiting in the second group of dogs was not observed in two cases and urination and defecation occurred in only two cases, whereas in the first group vomiting was always observed, and urination and defecation in 7 cases of 10.

Fewer changes of the condition of the heart were noted on EKG in dogs which received the combination of two preparations as compared with the first group, although these differences did not achieve statistical significance. Disturbances of the rhythm of heart contractions (atrial fibrillation), induction of myocardial excitation (incomplete atrio-ventricular block), and signs of myocardial ischemia (inversion of T wave and elevation of the ST segment) were noted in six dogs of the first group. Myocardial ischemia was noted in two dogs in the second group.

Influence of unithiol on the cystamine induced changes of pulse rate after 30 and 60 minutes of observation was not statistically significant, as shown in Table 2. Restoration of these characteristics to the initial level in dogs of both groups took place within the same time interval, one

hour after administration of the preparations. On the other hand, the changes in respiratory rate at the 30^{th} minute of observation in dogs of the second group were less pronounced, as depicted in Table 2.

SECTION 4

DISCUSSION

The results of these experiments show that cystamine, in the dose applied, expresses a toxic effect in animals, which is in complete accordance with literature data (see the Introduction). This dose provides protection of dogs against ionizing radiation (32). Because of this, the study of the antitoxic effect of unithiol is of interest for such conditions.

From Table 1 it will be obvious that the use of unithiol does not exclude toxic effects of cystamine in animals. They manifested themselves in all dogs which received cystamine, regardless of whether it was combined with unithiol or not. The decreased frequency of certain symptoms of intoxication after administration of unithiol (urination and defecation, EKG changes, and depression after agitation) was not shown to be significant by statistical analysis.

However, the antitoxic effect of unithiol is evident when other criteria are used; it is manifested by less expression of cystamine intoxication in dogs of the second group. In particular, this is reflected in the decreased duration of agitation in dogs which have received unithiol before cystamine: although onset occurs at the same time in animals of both groups, the period of agitation lasts half as long in dogs which have received the combination. Note that the agitation in these animals is never succeeded by depression.

The administration of unithiol has an effect on expression of the vomiting reaction; the average number of episodes of emesis and the duration of the period of emesis in dogs which have received cystamine with unithiol is significantly less than in the first group.

The antitoxic effect of unithiol manifests in more rapid cessation of all external manifestations of intoxication by cystamine as well; the animals in the second group return to normal far faster than those in the first. All this leads to the observation that for the most part it is possible to distinguish between animals in both groups by appearance and behavior, especially at the end of the first 30 minutes of observation. A decrease in the toxic effects of cystamine on breathing is evident as well.

The influence of cystamine administration in dogs on their physical work capacity is noted to be distinct from the reaction of rats: a dose of 60 mg/kg of the protector lowers the work capacity of the latter by 70% (14a). Apparently this discrepancy of the results is attributable to the fact that in our experiments the physical work capacity, because of peculiarities of method, was not evaluated at the peak of intoxication, but during the period of its decrease and disappearance, whereas the decrease of the work capacity in rats was tested just at this peak. A difference between the species in their reaction to cystamine is possible as well.

Thus these experiments show antitoxic effects of unithiol to cystamine in dogs. It is manifested in the differences of these effects in dogs which have received the cystamine radioprotector alone, vs. its combination with unithiol. The differences consist not in decrease of frequency of the toxic disturbances, but in reduction of the intensity of their expression.

Studies of the weakening of the toxic effects by thiol protectors in dogs are isolated. A favorable influence of pyridoxine decreasing the frequency of cystamine-induced emesis has been shown (44a). A combination of cysteamine with aminazine does not decrease the toxic effect of

cysteamine (35). In this connection, our results of the use of the combination of cystamine with unithiol may be considered encouraging.

The antitoxic effect of unithiol in dogs has been sufficiently demonstrated, though not to as impressive an extent as in mice and rats. In lethality studies the addition of unithiol to cystamine has raised the survival of the "cystamine" animals (15, 15a, 15b, 22). This is attributable to the qualitative difference of indicators used to ascertain the effect of unithiol. An "either/or" indicator, death versus survival, was used with rodents. However, in dogs indicators that varied in magnitude were used: more versus less, longer versus shorter. Obviously, even a small effect in the former case is more impressively demonstrated than a relatively greater shift in the second case. In addition to that, the ratio of doses of unithiol and cystamine which was optimal for decrease of the toxic effect of the protector in mice was used for this study in dogs. However, this ratio of doses for dogs could differ considerably from optimum in view of the species peculiarities of animals, as well as the different effects that were studied in mice. It is not improbable that for a different ratio of doses of the radioprotector and unithiol, the antitoxic effect of the unithiol would be more pronounced.

Further directions for research should include study of the antitoxic effect of unithiol with respect to cystamine in dogs using other ratios of doses of the radioprotector and unithiol. Appropriate indicators of effectiveness would be the frequency and duration of episodes of emesis. These indicators may be considered the main criteria of manifestation of cystamine intoxication. In such experiments it is possible to try to reduce the dose of unithiol, and still enhance the antivomiting effect by the addition of pyridoxine. The antitoxic effects of other dithiols with respect to thiol radioprotectors should also be studied.

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